

The Role of Auxin and Other Exudates in Mycorrhizal Symbiosis of Forest Trees

VISVALDIS SLANKIS

Seldom has a topic in botany aroused as much controversy as that surrounding the problem of ectotrophic and ect-endotrophic mycorrhiza of forest trees. As early as 1885, when Frank (15) in Germany first observed the abundance of mycorrhizae among forest trees and postulated that mycorrhizal fungi are symbionts indispensable to trees, strong opposition was aroused among several contemporary botanists who thought that, in the mycorrhizal associations, the fungus acts as a parasite. Although this opinion continued to reappear in the literature for the next fifty years, experimental data and field observations accumulated during the last three decades have provided evidence that this symbiosis is mutual, that is, beneficial for both symbionts. Furthermore, it has been found that when the tree and the fungus are not in symbiosis, they can under certain conditions suffer from nutritional deficiencies which result in severe growth disturbances.

It has been demonstrated that if mycorrhizal fungi are not in association with tree roots, they are not capable of producing fruiting bodies (Romell, 66). The ability of the fungus to accomplish its full life cycle only when in symbiosis with tree roots is an indication that some important substances in which the fungus is deficient or partially deficient are obtained from the roots of the higher symbiont. Extensive studies on the nutritional requirements of the lower symbiont carried out by Melin and his students under laboratory conditions have revealed that most of the mycorrhizal fungi lack the ability to produce cellulase and therefore are unable to utilize cellulose as a source of carbohydrates (33, 41, 42, 61). Only a few excep-

tions have been found (61, 33). All species of mycorrhizal fungi tested have proved to be at least partially thiamin deficient (45, 50, 52, 53, 55, 61). Some fungal associates have been found partially deficient in pantothenic acid and nicotinic acid (61), biotin (52, 55), and inositol (45). Amino acids have been reported as being significant to the growth of mycorrhizal fungi (46, 51, 55, 62, 44).

In assessing the benefit of mycorrhizal symbiosis to the tree, Frank pointed out that hyphae of the lower symbiont act as a nutrient-absorbing organ so that trees with mycorrhizae, according to Frank's theory, are better supplied with nitrogen. Later Stahl (71) published his "mineral theory" in which he claimed that in soils poor in mineral nutrients, trees are brought into strong competition with fungi and bacteria; nevertheless, through mycorrhizal symbiosis the tree is benefited. Melin (41), having accepted the theory of Stahl as a principle, assumed that in addition to inorganic nutrients, organic nitrogen compounds may also be utilized by mycorrhizal fungi.

Many recent experiments have provided evidence for these assumptions. Chemical analyses have shown that plants with mycorrhizae contain considerably more nitrogen, phosphorus, and potassium than plants without mycorrhizae (24, 57, 37). The accumulation of a larger quantity of phosphorus in mycorrhizal than in nonmycorrhizal roots has been demonstrated by Kramer and Wilbur, using labeled phosphorus (31). The transfer of labeled inorganic phosphorus into the roots by way of the hyphae has been proved by Melin and Nilsson (47), Harley and McCready (22, 23), and Harley and Brierley (21). The transfer of labeled inorganic nitrogen and organic nitrogen in the form of glutamic acid has been demonstrated by Melin and Nilsson (48, 49). Moreover, it has been proved that mycorrhizal symbiosis is of decisive importance in the afforestation of forestless areas in different parts of the world: West Australia (29, 87), Java and Sumatra (65), South Africa (3), Philippines (63), and certain prairie regions in North America (24, 85).

Since mycorrhizal symbiosis is widespread and habitual among forest trees, interest in this symbiosis has steadily increased in silviculture. In 1956 a working group was established at the International Union of Forest Research Organizations. This working group, studying practical problems of mycorrhiza, will strive to obtain a clear picture of this symbiosis under natural conditions and to determine means of controlling it for the maximum benefit of trees.

Aside from its practical aspect, a consideration of this symbiosis has continued to present a challenge to many workers as a biological

problem in which the relationships and the interdependence between fungus and tree is of great interest. The progress and the achievement in mycorrhizal research has been recently summarized in several comprehensive reviews (64, 25, 19, 20, 6, 28, 43). Though several hundreds of papers have been published in this field, the real mechanism of this symbiosis is still not fully understood. There is a most serious gap in information about the factors governing the formation and establishment of ectotrophic and ect-endotrophic mycorrhiza.

According to Hatch (25) mycorrhizae are produced in abundance under conditions of low availability or lack of balance in the availability of any one, or more, of these four elements: nitrogen, phosphorus, potassium, and calcium. Björkman (4, 5) found that, for the formation of mycorrhizae, light is of paramount importance and that only available nitrogen and phosphorus, acting in combination with light, exert a dominating influence. Having obtained experimental evidence of a correlation between the amount of soluble carbohydrates in pine roots and the frequency of mycorrhizae, Björkman postulated that mycorrhizal fungi parasitize the roots for soluble carbohydrates. According to his theory, the hyphae of the fungal associate enter into the roots and produce mycorrhizae only when the roots contain a surplus of soluble carbohydrates. Levisohn (32), however, has asked how the mycelium, before entering the roots, "knows" whether or not there is an excess of soluble sugars present in the roots. This question still remains unanswered.

Evidence recently obtained indicates that substances exuded by roots and by fungal hyphae play an important role in the initiation of mycorrhizal symbiosis. Although information about the role of fungous metabolites in the mycorrhizal relationship is still limited, it has been shown that the hyphae of mycorrhizal fungi exude auxin. McDougal and Dufrenoy (38) carried out extensive cytochemical studies of mycorrhizal pine roots and found auxin to be abundant in the hyphae of the lower symbiont. They assumed that this auxin is translocated into the roots and is responsible for the coralloid branching of short roots. The writer's recent studies have provided experimental evidence for this assumption. When excised Scots pine roots cultivated under aseptic conditions were inoculated with mycorrhiza-producing fungi (*Boletus luteus*, *Boletus variegatus*), the mycelia of the fungus induced, in the short roots, swellings that resembled the swellings of simple mycorrhizae. Many of these swellings soon exhibited a profuse dichotomous branching similar to that of coralloid mycorrhizae of pine. As a rule, root hairs were

absent on the swollen parts. Similar dichotomy was induced in roots by the addition to the root nutrient solution of a cell-free culture medium in which the fungus had been cultivated (67).

These results provide evidence that the characteristic morphology of simple and coralloid mycorrhizae is induced by some substance or substances exuded by the lower symbiont. These substances must be related to auxins since synthetic auxins, such as indoleacetic acid, indolebutyric acid, indolepropionic acid, and α -naphthaleneacetic acid, when added at certain concentrations to the nutrient solution of roots, induce similar morphogenesis, such as monopodial and dichotomously branched swellings resembling simple and coralloid mycorrhizae, and swellings resembling tubercle mycorrhizae (68, 69, 70). As in mycorrhizal roots, root hairs are absent on swollen parts and their cortical cells are hypertrophied. Moreover, by applying synthetic auxins at different time intervals it is possible to induce in pine roots several other morphological changes characteristic of mycorrhizal roots, such as prolonged renewed growth and intermittent growth (unpublished data).

It is still not known what kind of auxin is exuded by the lower symbiont. We may assume that it is indoleacetic acid, since we know from Nielsen (58, 59), Boysen Jensen (9, 7, 8), Kögl and Haagen-Smit (30), Dolk and Thimann (12) and Thimann and Dolk (80) that numerous microorganisms such as bacteria and fungi are able to produce indoleacetic acid in considerable amounts in culture. We also know that large amounts of indoleacetic acid, produced by the invader, have been detected in the tissues of legume nodules (34, 35, 76, 78) and crown galls (36).

Data obtained by several workers (2, 10, 13, 14, 17, 75) confirm that, of plant organs, roots are the most sensitive in their reaction to the auxin and that their elongation is accelerated only at extremely low auxin concentrations. Higher concentrations inhibit the elongation of the roots, an inhibition which is accompanied by swellings of the root tips due to the isodiametric growth of root cells. Since the monopodial and dichotomously branched swellings of pine roots can be obtained only at relatively high auxin concentrations (68, 69, 70), these forms should be considered as resulting from the inhibited elongation of the roots. However, if the addition of auxin is discontinued, the elongation of the swollen root tips is resumed in a few days. The newly grown parts are not swollen and their distal region is often covered with root hairs (70).

A great similarity in morphogenesis is observed in pine roots under natural conditions during their conversion into mycorrhizae. This conversion is always accompanied by monopodial or dichotomously

branched swellings of the root tips and an absence of root hairs on the swollen parts (Figs. 21-1 and 21-2). Mycorrhizal roots often renew their elongation. When the renewed growth continues for an extended period, the newly formed part of the root is relatively long, unswollen and develops root hairs on its distal end. Since the similarity of root morphogenesis is so great, whether induced by high concentrations of synthetic auxins or auxins exuded by mycorrhizal fungi, it may be concluded that the characteristic morphology of mycorrhizal roots results from the inhibition of their elongation, induced by high concentrations of auxin exuded by the lower symbiont. Because a swelling is an essential morphological element in pine mycorrhiza, it can be postulated that symbiosis functions in mycorrhizal roots only as long as the lower symbiont is capable of producing and translocating auxin into the root tip at

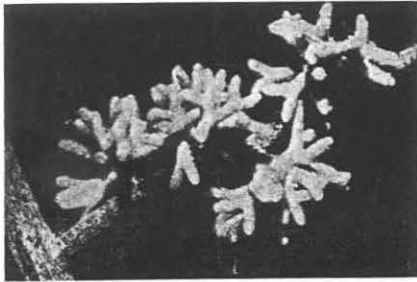


FIG. 21-1. Coralloid mycorrhizae of *Pinus strobus* L. (ramifying hyphal threads removed). $\times 5$.

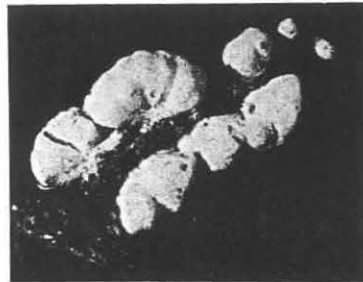


FIG. 21-2. Tuberous mycorrhizae of *Pinus strobus* (ramifying hyphal threads removed). $\times 5$.

concentrations which markedly inhibit its elongation. The deviations in form of mycorrhizal roots are not, however, permanent alterations. If, for some reason, the lower symbiont loses its capacity to produce auxin in the required concentration, the roots resume their elongation and the mycorrhizal relationship ceases to exist.

Melin (39, 40), after extensive cytological studies of mycorrhizal roots, concluded that a deep intracellular infection of the hyphae occurs in the cortical cells in the initial stage of the formation of the symbiosis. With increasing resistance of the host plant, however, most of the intracellular hyphae are digested by the host cells; as a result, in a later stage, the hyphae of the aggressor occur mainly intercellularly, where they assume a specific configuration known as the "Hartig net." Melin regards the presence of the Hartig net as an indication that a mutual relationship has been established in the mycorrhizal root. Several other investigators also consider the fungus to be an aggressor and that the mutuality in the symbiosis de-

depends upon the ability of the host plant to contain the fungus within certain limits. Melin (43) has recently expressed his opinion of the nature of the fungal symbiont as follows: "Since the mycorrhizal fungus invades the host, it may be considered primarily as a parasite. Contrary to many real parasites it does not seem, as a rule, to be producing substances toxic to the host."

In our experiments with excised and intact pine roots, it became evident that there is a close relationship between the degree of deviation in root morphology and the concentration of auxin applied. Thus, forms resembling simple mycorrhizae can be reproduced with relatively low concentrations of indoleacetic acid (1 to 2 mg. per l.); coralloid branching, especially profuse coralloid branching, with higher concentrations (5 to 10 mg. per l.); while forms resembling

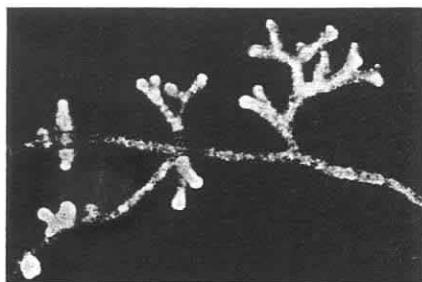


FIG. 21-3. A fragment of excised root of *Pinus sylvestris* L. with dichotomously branched short roots. Dichotomy induced by naphthaleneacetic acid 2.5 mg. per l. $\times 5$.



FIG. 21-4. A fragment of excised root of *Pinus sylvestris* with extremely swollen short roots. Tubercle-like swellings induced by naphthaleneacetic acid 10 mg. per l. $\times 5$.

tubercle mycorrhizae are obtained only when the concentration of auxin is relatively high (10 to 20 mg. per l.). These differences in form are illustrated in Figs. 21-3, 21-4, and 21-5. It should be stressed that these forms are induced by auxins at concentrations that strongly inhibit the elongation of the roots and which, according to Gautheret (16), far exceed the limit of the range of physiological action of auxin.

From these results we may infer that under natural conditions the degree of morphogenetic deviation in mycorrhizal roots depends upon the concentration of auxin exuded by the fungus. Mycorrhizal root morphology must be considered as superimposed upon the higher symbiont through the influence of these auxins which cause the striking growth deviations of these roots: swellings, profuse branching of coralloid mycorrhizae, the extremely compact and swollen short roots of tubercle mycorrhizae, and the absence of root hairs on the swollen parts.

How should these deviations be regarded? One might ask if their morphology exhibits any pathological symptoms, especially in those cases in which the morphological deviations have reached their maximum expression, as is the case in tubercle mycorrhizae. Thimann (84, 79) classifies the nodules of leguminous roots as pathological formations, though the symbiosis itself is considered beneficial to the host plant. This may provide some background for our present discussion. According to his theory (76), a nodule is induced on a secondary root which has been strongly inhibited by the auxin produced by *Rhizobium*. The morphology of mycorrhizal roots also re-

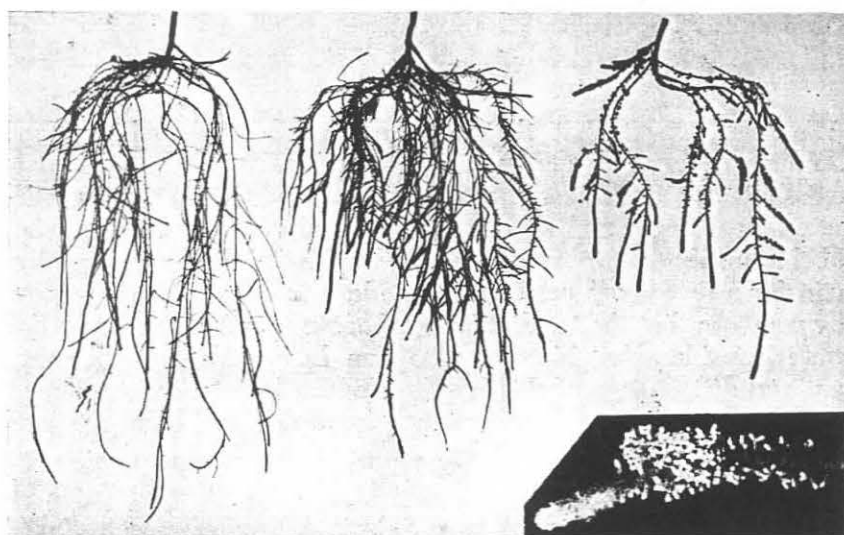


FIG. 21-5. Root systems of eight-months-old seedlings of *Pinus strobus* cultivated in nutrient solutions under aseptic conditions. *Left*—control. *Middle*—indoleacetic acid added, the amount of which is gradually increased from 0.0005 mg. to 0.045 mg. per l. *Right*—indoleacetic acid added, the amount of which is gradually increased from 0.0005 mg. to 9 mg. per l. $\frac{1}{4}$ natural size. *Insert*—magnified tip of long root with dichotomously branched short roots from root system right, above. $\times 2$.

sults from growth inhibition of rootlets due to auxin exuded by the lower associate. Therefore, a similar conclusion, based not on function but only upon variations in form, would be that these morphological patterns display pathological deviations.

On the other hand, we may assume theoretically that the lower symbiont under certain conditions can produce auxins at concentrations which distort the balance of normal cell physiology. In such a case, a point would be reached at which the lower symbiont no longer acts as a mutual associate, but begins to be the dominant partner in the symbiosis. The literature does not record recent investigations to suggest that any pathological condition is

exhibited in the relation between the host and the lower symbiont in the simple and coralloid mycorrhizae after the symbiosis has been established. It may be, however, that pathological symptoms can be traced in the case of tubercle mycorrhizae. That this type of mycorrhiza retains its hyphae intracellularly for a prolonged period has been confirmed by several investigators. The deep intracellular infection of cortical cells indicates that the capacity of these cells to restrict invasion is diminished, and we may assume that the relationship has been at least slightly modified in favor of the lower symbiont. In view of our incomplete information it would be unwise to conclude that tubercle mycorrhiza exhibits true pathological symptoms. We may, however, assume that in this type of mycorrhiza a threshold is reached beyond which real pathogenicity is encountered.

We may expect that the auxin exuded by mycorrhizal fungi would not be strictly confined in mycorrhizal roots but would be partially translocated into the long roots. Taking into consideration that a root system usually has numerous well-developed mycorrhizae and that under certain conditions the hyphae of the fungus produce auxin for a prolonged period and exude it in mycorrhizal roots, we may presume that the total amount of auxin translocated from these roots is considerable. Nothing is known, however, about the movement and distribution of this fungus-produced auxin, or its influence on the root system. It is known that auxin does not exhibit a strict one-way polarity in roots. The movement of auxin in roots can take place acropetally (77), basipetally (11), or in both directions (18, 26). If the auxin translocated from mycorrhizal roots moves into the mycorrhizae-bearing long root basipetally, then it can be expected to enter into the pioneer roots,* and through these to spread into the whole root system. Because of the large difference in volume between the mycorrhizal roots and long roots, the concentration of this auxin in the long roots would be relatively low. If, on the other hand, part of this auxin is transported acropetally, then, according to the "band theory" of Went and Thimann (84), this auxin will accumulate in the distal part of the mycorrhizae-bearing long roots. Experiments in which synthetic auxins were applied to excised (70) and intact pine roots (unpublished data) have shown that low concentrations of auxin markedly stimulate the elongation of long roots. Relatively high auxin concentrations, on the contrary, inhibit the elongation of long roots and induce swellings on their distal parts. On these swollen parts numerous new

* Following Noelle (60) and Aldrich-Blake (1) pioneer roots are the most poorly branched long roots of pine which elongate rapidly and extend out into the soil.

roots are initiated which, as a rule, are already swollen when they emerge through the epidermis. In both cases, the long/short root ratio is considerably changed and as a result the pattern of the root system changes. These experiments, however, do not provide any information about auxin transportation and distribution in the roots, since auxins were applied externally to the whole root system.

Recently, the movement in pine roots of auxin derived from an external source was demonstrated (unpublished data). Intact roots of white pine were cultivated under aseptic conditions in specially designed culture tubes. These tubes were divided into three compartments by means of thresholds which allowed the addition of auxin to either the basal, middle, or distal part of the root system. In order to observe the auxin movement in the roots and its eventual influence in different parts of the root system, indoleacetic acid was added to the middle part of the root system (the area of which is about one-third of the whole root system). It was found that this auxin, at a concentration which began to inhibit the elongation of roots in the middle compartment, induced an intensive elongation of roots in the other two compartments. This resulted in a remarkable increase in the long/short root ratio in both proximal and distal parts of the root system. These rapidly elongating roots have only a few laterals and the diameter of these roots is larger than that of long roots from the control plants. In general the appearance of these elongating roots resembles that of pioneer roots of pine. When the concentration of auxin in the middle compartment was gradually increased, an inhibition of root elongation in the other two compartments followed. As a result of this inhibition relatively long and clavate swellings appeared on the long roots and many new short roots were initiated on the swollen region. These new roots appeared to be already swollen as they emerged from the epidermis of the long roots. These long roots with slender clavate swellings and bearing a large number of swollen short roots resemble, in general, the similarly shaped long roots with numerous mycorrhizae that are frequently found under natural conditions. The inhibiting effect, however, appeared much earlier and was more strongly expressed in the distal than in the proximal part of the root system.

These results indicate that, of the auxin absorbed in the middle part of the root system, a certain amount is translocated in each direction. However, the stronger inhibiting effect observed in the distal part of the root system indicates either that more auxin has been transferred acropetally than basipetally, or that auxin transferred acropetally accumulates in the root apices.

The striking similarity between the morphogenesis of long roots induced experimentally with synthetic auxins and the morphogenesis of long roots observed under natural conditions suggests that fungus-produced auxin is transported in both directions in long roots and, depending upon its concentration, determines the degree of morphogenesis of these roots.

Thus, it appears that the auxin exuded by the fungus plays a multifarious role in the roots:

1. It causes all the morphological deviations of short roots which are essential to the establishment of ectotrophic and ect-endotrophic mycorrhizae of pine.
2. It influences the whole root system when translocated from mycorrhizal roots into the long roots. Depending upon its concentration, this auxin stimulates or inhibits the elongation of long roots. If the growth inhibition is strong enough, numerous new roots are initiated.
3. It determines broadly the sequence and frequency of short and long roots and thereby the pattern of the root system.

However, the information obtained about the role of fungus-produced auxin in mycorrhizal symbiosis is still limited. So, for instance, we have no information about the role of this auxin in the cell metabolism of roots and its influence on the growth of the entire tree. Furthermore, despite the fact that the Hartig net, as a rule, is found only in swollen roots in which the cortical cells are hypertrophied, we do not know why this anatomical change is a prerequisite for the establishment of ectotrophic and ect-endotrophic mycorrhizae. Obviously, these deviations in anatomy indicate a fundamental change in the physiology of the cortical cells.

Since auxin (or auxins) exuded by the fungal symbiont is so deeply involved in the mechanism of mycorrhizal symbiosis, emphasis should be placed upon a study of the factors that determine the production of auxin in the hyphae. Undoubtedly the production of auxin by the fungus depends on the nutrient available from the soil and especially from the tree roots. We may presume that root metabolites, particularly amino acids, condition the auxin-producing potentiality of the fungus. It has been reported that microorganisms convert certain amino acids (8) but particularly tryptophane (74) into indoleacetic acid.

In 1923 Melin introduced the term "virulence" of the mycorrhizal fungus. He pointed out that if the fungus is not virulent enough, the symbiosis does not become established even though the hyphae have invaded the roots. We may interpret "virulence" as the ability of the fungus to produce auxin in concentrations that will

ensure the degree of morphogenesis necessary for the establishment of mycorrhizal symbiosis.

In a discussion of the factors that may determine mycorrhizal symbiosis, the role of root metabolites must not be neglected. Obviously, root metabolites compensate for the nutritional deficiency to which the fungus is exposed when not in symbiosis with tree roots. How otherwise can we explain the fact that mycorrhizal fungi are not able to complete their full life cycle when they are not associated with tree roots (66)? Recently it has been found experimentally that root metabolites, other than soluble carbohydrates, largely restrict the effectiveness and action of the auxin in the roots (unpublished data).

If root metabolites are exuded into the rhizosphere, they can influence the fungus before the invasion of roots occurs. We may assume that the root exudates in the rhizosphere induce a chemotropic response in these fungi. It has been shown that the rhizosphere of different herbaceous plants is inhabited by an exceptionally rich microflora (27, 72, 73, 81). It has been thought that the excretion of water-soluble compounds by roots was the chief cause for the increase in the microbial activity of the rhizosphere. Winter (86), experimenting with several cereal plants, has provided evidence that this increased microbial activity in the rhizosphere is caused by exudates from the roots.

That roots of pine exude various organic substances has been clearly demonstrated. On several occasions, when nutrient solutions in which intact pine roots growing under aseptic conditions were inadvertently contaminated by airborne fungi, the occurrence of a vigorous mycelial growth has been observed even though the solutions contained only inorganic salts (unpublished). Experiments with excised pine roots inoculated with mycorrhiza-producing fungi demonstrate that the growth increment of the mycelium is much higher in the presence of roots (67, 44). This indicates that pine roots exude certain metabolites which are of vital importance to the growth of the fungus. Recently, using isotope techniques, it has been proved that the roots of pine seedlings exude large amounts of different organic substances. In these experiments, the needles of the white pine seedlings were fed with labelled $C^{14}O_2$. Using electrophoresis and chromatography, it was found that in a period of eight days the roots of the seedlings exuded a large number of radioactive organic substances. Although the final identification of these substances has not been completed, preliminary analyses reveal the presence of sugars (glucose and arabinose), a large number of different amino acids, and large quantities of organic acids (unpub-

lished data). This indicates that, in addition to soluble sugars, several other substances of vital importance to the fungus are present in the roots and, under certain conditions, are exuded.

These results may help us to understand the theory, as stated by Björkman, that hyphae seek to enter the roots only when the roots contain surplus sugar. First, we may assume that some of this excess sugar is exuded into the rhizosphere. Since we know that mycorrhizal fungi require soluble carbohydrates, the presence of sugars in the rhizosphere may stimulate the hyphae to grow toward the roots and finally to invade the sugar-rich cells. The sugars, however, under our experimental conditions, were exuded in relatively small amounts compared to the amounts of the other compounds mentioned above. If similar conditions occur generally, it seems more logical to assume that the actual stimulating factors in the root exudates, which are of vital importance to the growth of the fungus, are amino acids, vitamins, or other specific substances, the nature of which is still unknown [cf. Melin's "M" factor (54)]. We can expect that every change in the environmental condition of the host plant influences, quantitatively and eventually qualitatively, the composition of the root metabolites, and subsequently the root exudates. We may assume that when surplus sugar occurs, the roots may exude metabolites into the rhizosphere, which stimulate the hyphae to grow toward and ultimately to invade the roots.

Undoubtedly, root exudates of the higher symbiont play a much broader role in mycorrhizal symbiosis than the simple stimulation or inhibition of the growth of the hyphae of the fungal symbiont. It is well known that these exudates greatly influence the activity of the microflora, including mycorrhizal fungi, in the rhizosphere. These microorganisms, undoubtedly, produce and exude specific substances of their own. We may presume that the antibiotic and synergistic action induced by exudates, between roots and the rhizosphere-inhabiting microorganisms and at the same time between the microorganisms themselves, largely determines the composition of microflora in the rhizosphere. Mišustin (56) and Tribunskaja (83) found that the population of fungi and bacteria was much higher in the rhizosphere of tree seedlings with mycorrhizae and that when tree seedlings with mycorrhizae were inoculated with *Azotobacter*, they exhibited better growth and increased mycorrhizal development.

It is very apparent that our knowledge of the roles of metabolites exuded by roots, mycorrhizal fungi, and other soil-inhabiting microorganisms of the rhizosphere must be extended. In a broad investigation of the rhizosphere we should search for the keys that will

unlock the doors to a more complete understanding of the mysteries of tree growth and mycorrhizal symbiosis.

Discussion

LEYTON: In cultures of excised Scots pine roots in humus extracts, in the absence of any added auxin, we did manage to get forking of the roots. This raises the possibility that free auxin is present in the humus. Clowes has also obtained forking of cultured pine roots in the absence of either auxin or humus. In one of our heathland experiments, scalping off the heather vegetation was followed immediately by the appearance of a considerable number of sporophores of *Boletus*; subsequently we found that the trees growing into these areas became mycorrhizal. Further experiments, in which I have collaborated with Dr. Levison, have shown that this mulching treatment, which very effectively stimulates tree growth, is almost invariably followed by pronounced mycorrhizal infection of the roots.

SLANKIS: You referred to dichotomously branched roots in media to which no auxin was added. Were the branches of these forks actually swollen in the manner of coralloid mycorrhizae?

LEYTON: In the humus extract they were slightly swollen.

SLANKIS: Possibly the dichotomy which you and Dr. Clowes observed was caused by conditions unfavorable to the growth of the roots. I have observed similar phenomena when the growth of the roots was inhibited for a certain period by suboptimal nutrition or by superoptimal temperature. It is of interest that an intensive dichotomous branching is induced in a few days when the growth-inhibiting factors are eliminated. In this case, however, as a rule no repeated dichotomy is observed and the branches of the forks gradually elongate and lose the characteristic morphology of coralloid mycorrhizae.

HUBER: Have you made any observations on bleeding in pine roots, about which there has been some controversy?

SLANKIS: Roots grown in an auxin medium are much more turgid than the control roots. When auxin-treated roots are cut, there is rapid exudation of a few droplets.

RICHARDSON: Have these fungal exudates ever been applied to other parts of plants, such as *Avena* coleoptiles?

SLANKIS: Not as far as I know.

RICHARDSON: Could it be that the fungal exudate is actually activating auxin that is already present in the root? Before I attended this meeting, I had assumed that the leaves of my *Acer* seedlings were supplying a growth factor other than auxin, which, together with auxin, was giving normal root development. When the seedlings were grown in soil instead of water culture, the fact that leaves did not appear to be necessary could be explained by hypothesizing that the soil was supplying this other factor. I was thinking in terms of vitamins. Now I wonder if auxin alone is in-

volved and if the role of the leaves is somehow to reduce the effective concentration in the roots, and the role of the soil possibly to do the same.

LEYTON: It is not impossible, is it, that the microorganisms in the soil produce auxin?

THIMANN: In fact we have shown this to occur, but the amounts are very small and extremely erratic. Sometimes, under what appeared to be perfectly good conditions, we found no auxin and at other times we obtained clear indications that auxin was present.

Dr. Slankis mentioned the large differences in auxin concentration needed for mycorrhizal formation, compared with those effective in tissue cultures, as listed by Gautheret. Tissue cultures respond to lower levels of auxin than intact plants. In shoot cultures and root cultures the difference between the effective concentrations is of the order of five or ten times. One has to watch the arithmetic, however, because the French workers express auxin concentrations in parts rather than molarity. When they refer to an IAA concentration of 10^{-7} they do not mean 10^{-7} molar, they mean 10^{-7} parts per part. There is a six-fold difference between the two.

In your pictures I was impressed again by the similarity between mycorrhizae and nodules in legumes. This is, of course, an old observation. However, we know now that *Alnus* nodules do fix nitrogen and it was shown many years ago that the nodules of *Coriaria japonica* do fix nitrogen also (their organisms apparently are not Rhizobia). These observations raise again the old question of the ability of mycorrhizae to fix nitrogen. I think with modern methods and your sterile culture techniques this question might be worth resuscitating.

SLANKIS: Melin has concluded that there is no nitrogen fixation by mycorrhizae. Nevertheless, considering the wide range of mycorrhizal fungi, it might be held that the few species examined by Melin should perhaps be augmented.

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